

# PESQUISAS

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## ECOLOGICAL AND REPRODUCTIVE ASPECTS OF *Syngonanthus caulescens* RUHLAND (ERIOCAULACEAE) IN SÃO FRANCISCO DE ASSIS, RIO GRANDE DO SUL STATE, BRAZIL

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### ABSTRACT

*Syngonanthus* (Eriocaulaceae) occurs in American and African continents and *Syngonanthus caulescens* is the most widely distributed species of this genus. Our research aimed to evaluate the reproductive capacity and floral visitors of two populations in São Francisco de Assis (RS), ecological niche modelling of *S. caulescens* and possible implications for conservation. We analyzed meiotic index, pollen viability and the vegetative propagation capacity, complemented by observations on floral visitors and ecological niche modelling. The results showed irregular meiotic behavior and low pollen viability, in opposition to a wide vegetative propagation capacity. The main group of floral visitors in these populations was Diptera, but other orders of Insecta, besides specimens of Arachnida, were also found. The analysis of the potential geographical distribution considered the Espinhaço Range and Brazilian coast as the most favorable sites for the occurrence of *S. caulescens*, although this species is widely distributed throughout Brazilian territory, as well as in Bolivia and Venezuela, where it occurs in very specific environments. In this way, it is possible to delineate conservation strategies for the species by emphasizing protection of the ecosystems where it occurs, which are under strong threat of agricultural expansion, such as the Pampa.

**Key words:** Ecology, Reproduction, Wetlands.

### RESUMO

*Syngonanthus* (Eriocaulaceae) ocorre nos continentes americano e africano e *Syngonanthus caulescens* é a espécie mais amplamente distribuída deste gênero. A pesquisa objetivou avaliar a capacidade reprodutiva e os visitantes florais de duas populações em São Francisco de Assis (RS), modelagem de nicho ecológico da espécie *S. caulescens* e possíveis implicações para a conservação. Foi analisado o índice meiótico, viabilidade polínica e capacidade de propagação vegetativa, complementados

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por observações dos visitantes florais e modelagem de nicho ecológico. Os resultados mostraram comportamento meiótico irregular e baixa viabilidade polínica, em oposição a uma ampla capacidade de propagação vegetativa. O principal grupo de visitantes florais foi Diptera, mas outros ordens de Insecta, além de espécimes de Arachnida, também foram encontrados. A análise de distribuição geográfica potencial considera a Cadeia do Espinhaço e a Costa Brasileira como os locais mais favoráveis para a ocorrência de *S. caulescens*, embora esta espécie seja amplamente distribuída ao longo de todo o território brasileiro, além da Bolívia e Venezuela; ocorre em ambientes específicos. Desta forma, é possível delinear estratégias de conservação para a espécie enfatizando a proteção dos ecossistemas onde ela ocorre, os quais estão sob forte ameaça com a expansão da agricultura, como o Bioma Pampa.

**Palavras-chave:** Ecologia, Reprodução, Áreas úmidas.

## INTRODUCTION

Eriocaulaceae Mart. is widely distributed in the tropics and subtropics, comprising about 1,400 species in 10 genera (Giulietti *et al.*, 2012). The main diversity center is the Espinhaço Range, in Minas Gerais State, Brazil (Sano *et al.*, 2015). 96% of the Brazilian species of Eriocaulaceae are endemic (BFG, 2015; Sano *et al.*, 2015), growing exclusively in sandy and humid soils (Trovó *et al.*, 2015; Demetrio & Coelho, 2018). Echternacht *et al.* (2011) suggest that some species have a limited capacity for colonization, probably because the availability of its specific soil type. In association with endemism, the extractivism also contributes to the risk of extinction of many species. Eriocaulaceae are called “sempre-vivas” because their capitula keep living appearance even after removed (Giulietti *et al.*, 1996; Costa *et al.*, 2008), being exported and used in ornamentation. The disorderly extraction caused a drastic reduction of their populations and placed some species at the extinction risk (Costa *et al.*, 2008), mainly the *Syngonanthus* Ruhland species.

*Syngonanthus* comprises about 150 species in Americas and Africa (Echternacht *et al.*, 2014). *Syngonanthus caulescens* Ruhland has the widest geographical distribution and morphological variation within the genus (Watanabe *et al.*, 2017), being extremely important to extend knowledge beyond a morphological description. *Syngonanthus caulescens* is an herb with elongated stem and lanceolate leaves arranged equally along its entire length and white capitula, occurring in flooded field areas (Watanabe *et al.*, 2017). In the State Rio Grande do Sul, such areas are common in the grass savannahs called Pampa Biome, which is under agricultural expansions leading to loss of biodiversity and habitat fragmentation and transformation (Echer *et al.*, 2015). Survival and persistence of plant species in adverse environments requires strategies and phenological alternatives. *Syngonanthus* species can exhibit clonal growth associated to sexual reproduction with seed production dispersed over the entire year (Schmidt *et al.*, 2007; Bento *et al.*, 2010). This work aimed to analyze the reproductive strategies of *S. caulescens*, that allow their maintenance in the typical environment of the species, the flooded field, and to determine its potential geographical distribution to the Brazilian territory.

## MATERIAL AND METHODS

### Plant material

We analyzed two populations of *S. caulescens* growing on flooded field and sandy soil in the municipality São Francisco de Assis, Rio Grande do Sul State, Brazil. The

population one (P1; on the road BR 377, 29°35'67.9"S, 55°06'64.6"W) was considered as two subpopulations (P1A and P1B) in this study, because a stream divided it. The population two (P2) was located near the Miracatu River (29°35'16.4"S and 55°25'14.7"W). Vouchers were deposited in the Rogério Bueno Herbarium (HUIRB) of the Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUI), Ijuí, Rio Grande do Sul State, Brazil, under registration numbers HUIRB 7623 (P1A), HUIRB 7616 (P1B) and HUIRB 3882 (P2).

### Meiotic and pollen viability analysis

On December 16, 2016, randomly, about 30 young inflorescences of 10 individuals of each population and subpopulation (P1A, P1B and P2) were collected and immediately fixed in Carnoy 3:1 (ethanol: acetic acid) for 24 hours at room temperature, transferred to ethanol 70% and stored at 4°C. For meiotic and pollen viability analysis, the slides were prepared using the squashing technique of the anthers and colored with acetic orcein 2% (Guerra & Souza, 2002). For meiotic analysis, only the tetrad phase was observed, counted and classified according to the microspores number. The cell groups with only two microspores were called dyads, triads with three microspores, and polyads with number of microspores above five (Figure 1a). The meiotic index was calculated according Love, (1949):  $MI = \text{number of normal tetrads} / \text{total number of tetrads} \times 100$ , considering normal the tetrads with four equal cells and abnormal any other formation. To estimate the pollen viability, we considered viable the pollen grains which became stained and unviable the not stained, deformed or empty. Pollen viability was calculated by:  $PV = \text{number of viable pollen grains} / \text{total number of pollen grains} \times 100$ . We analyzed four slides of the anthers of each individual and phase (tetrads and pollen grains) from each population and subpopulation. The slides were analyzed under a Leica® optical microscope with the objective lens of 100x to observe the tetrads and 40x to the pollen grains. All results were submitted to  $\chi^2$  test ( $p < 0.05$ ) with BioEstat 5.0 (Ayres et al., 2007) to evaluate differences in the meiotic index and pollen viability among populations.

### Estimation of vegetative propagation capacity

We sampled randomly four quadrants measuring 10 cm x 10 cm in each population and subpopulation (P1A, P1B and P2), according with Bento *et al.* (2010) for *Syngonanthus chrysanthus* Ruhland. In each quadrant we counted all plants and the numbers of: a) clumps, b) isolated rosettes (considered those that had no connection with another plant), c) rosettes by clumps, d) vegetative rosettes and e) reproductive rosettes. All results were submitted to  $\chi^2$  test ( $p < 0.05$ ) with BioEstat 5.0 (Ayres *et al.*, 2007) to compare the results among populations and phenology stage.

### Floral visitors

Floral visitors were observed and sampled in approximately 1 m<sup>2</sup> in each population and subpopulation on October 23-25, 2017, during full flowering of *S. caulescens*. The floral visitors were observed in each population and subpopulation in intercalated periods of 30 minutes, one hour and 30 minutes in the mornings, from 8:00 am to 11:00 am, and afternoons, from 14:00 pm to 17:00 pm, totalizing three hours in each population and subpopulation. Floral visitors were photographed with a Nikon 7000, collected with tube and stored in ethanol 70% to posterior identification at the lowest possible classification level.

## Mapping and niche modeling

To construct a distribution map for the species, we used data collection (Table S1) containing original coordinates to *S. caulescens* (all the varieties) and synonyms (*Dupatya caulescens* Kuntze, *Eriocaulon caulescens* Poir, *Paepalanthus caulescens* (Poir.) Kunth, *Syngonanthus caulescens* var. *angustifolius* Moldenke, *Syngonanthus caulescens* var. *hirsutus* Moldenke, *Syngonanthus caulescens* var. *proliferus* Moldenke, *Syngonanthus glandulosus* var. *epapillosus* Moldenke and *Syngonanthus glandulosus* Gleason), available at Re flora Virtual Herbarium (<http://inct.florabrasil.net>). DIVA-GIS version 7.5 was used to produce the distribution map of *S. caulescens* (Hijmans et al., 2012). The potential distribution of *S. caulescens* was modeled with the Bioclim algorithm based on the 19 bioclimatic variables of the Worldclim database at a resolution of 2.5 minutes per pixel using version 1.3 (available at <http://www.diva-gis.org/Climate.html>). MaxEnt (version 3.3.3) was used to produce the map of potential geographical distribution, and to calculate the lowest presence threshold (LPT), Jackknife analysis, omission/commission errors and true skill statistic (TSS) (Phillips et al., 2017). The potential distribution map shows the environmental suitability with values ranging from 0-1 (0: low or no environmental suitable; 1: high or total environmental suitable).

## RESULTS

### Meiotic behavior and pollen viability

In the meiotic analysis, the tetrad phase was observed in all populations and subpopulations (Table 1). We found a total of 197 cell groups corresponding to the tetrad phase, considering all sampling areas. In the area P1A, 85 tetrads were observed, being 16 normal (with four microspores); in P1B, a total of 66, being 15 normal; and in P2, 46 being nine normal tetrads (Table 1). We found cells groups with numbers of microspores ranging from two to 11. The meiotic indexes were low in all populations and subpopulations, 18.8% in P1A; 22.7% in P1B and 19.6% in P2, not statistically differing from each other ( $\chi^2$ ,  $p = 0.8856$ , Table 1).

In the pollen analysis, a total of 2,080 pollen grains were observed in all populations (Table 2). In P1A, 453 pollen grains were observed, 313 viable and 140 unviable; in P1B, 1,080 pollen grains were observed, 768 viable and 315 unviable; and in P2, 544 pollen grains were found, 20 viable and 524 unviable (Figure 1b-c). In the subpopulations P1A and P1B, the pollen viabilities were high, 69.09% and 70.91% respectively, while in the population P2 it was low, 3.67%. There was no difference between the subpopulations P1A and P1B ( $\chi^2$ ,  $p = 0.7761$ ), however, both subpopulations differed from P2 ( $\chi^2$ ,  $p < 0.0001$ ) (Table 2).

### Vegetative propagation capacity

The vegetative propagation analysis sampled a total of 211 individuals (Table 3). In P1A, we observed the lowest number of clumps, only four clumps ( $\chi^2$ ,  $p = 0.0156$ ;  $p = 0.0219$ ), while P1B and P2 presented 18 and 17 clumps, respectively ( $\chi^2$ ,  $p = 0.9498$ ). The average number of individuals per clump did not differ among the sampled populations, 5.25 in P1A, 4.89 in P1B and six in P2 ( $\chi^2$ ,  $p = 0.88$ ). The lowest number of sampled individuals was observed in P1A, with 21 individuals in four clumps ( $\chi^2$ ,  $p < 0.0001$ ); between P1B and P2 there was no significant difference, 88 and 102 individuals, respectively ( $\chi^2$ ,  $p < 0.4495$ ).

In relation to the phenological phase, we quantified less individuals in reproductive stage than in vegetative stage in P2 ( $\chi^2$ ,  $p = 0.0009$ ), while in P1A more individuals were found in reproductive stage than in vegetative stage ( $\chi^2$ ,  $p = 0.0856$ ) and P1B had the same number in each phase, 44 individuals ( $\chi^2$ ,  $p = 0.0001$ ) (Table 3).

### Floral visitors

During the observation period, 29 individuals that visited the inflorescences belonging to Insecta and Arachnida were sampled (Table 4; Figure 2).

We observed specimens belonging to five orders of Insecta: Diptera (Figure 2a-c), Coleoptera (Figure 2d), Hemiptera (Figure 2e), Hymenoptera (Figure 2f) and Lepidoptera. Different families were observed in each Order, three families in Diptera (Callyphoridae, Muscidae and Syrphidae), one family in Hymenoptera (Formicidae), two families in Coleoptera (Crysolmelidae and Curculionidae), one family in Hemiptera (Psylloidae) and two immature individuals (larval phase) of Lepidoptera (Table 4).

All the specimens of Arachnida belong to the Suborder Araneomorphae. The classification in lower categories was not possible, because the specimens were immature (Table 4; Figure 2g-h).

### Potential distribution of the suitable environments

We obtained a total of 1,287 collections records of *S. caulescens* in the Re flora Virtual Herbarium, of which only 146 could be used in the analysis of the potential distribution of suitable environments because they contained the original coordinates (Table S1).

*S. caulescens* is widely distributed throughout Brazil, concentrating near the family diversification center, in the Espinhaço Range, Minas Gerais, and on the Brazilian coast (Figure 3a). There are no collection records for some States in the North and Northeast regions from Brazil.

The potential distribution of suitable environmental map agrees with the distribution map, indicating an average to low possibility of occurrence of *S. caulescens* in almost all the Brazilian territory, except the extreme South, and the North and Northeast Brazilian regions. High possibility of distribution is observed in the mountain ranges near to the diversification center. In addition, it is also possible to visualize favorable environments in Venezuelan and Bolivian territories (Figure 3b).

## DISCUSSION

According to our results, the sexual reproductive potential in *S. caulescens* is relatively restricted. Tetrads and pollen grains analyses demonstrated that the capacity to form meiotic products is far below expectations, an average 20.3% and 52.9% to meiotic index and viable pollen, respectively (Table 1 and 2). The meiotic index is a direct indicator of regularity meiotic division (Love, 1949) and widely used by several authors for this finality (Macedo *et al.*, 2017; Braga *et al.*, 2018; Zortéa *et al.*, 2019). The meiotic process can be considered normal if the plant has a meiotic index higher than 85% (Love, 1951). Abnormalities during meiotic division results in low meiotic index and pollen viability (Granato *et al.*, 2019). Pollen viability connotes the ability of pollen to complete post-pollination events and to effect fertilization, and among the most tests is the use of stains (Shivanna & Rangaswamy, 1992). Studies with other botanic families showed high meiotic index and pollen viability, as in *Hyptis mutabilis* (Rich) Briq., Lamiaceae, that presented meiotic index and pollen viability above 70% and 80% respectively (Fachinnetto &



Tedesco, 2009). *Bidens pilosa* L., Asteraceae, also presented high meiotic index and pollen viability, with values higher than 86% and 94%, respectively (Fachinetto et al., 2008). In Araceae Juss., the variation was of 88 to 100% in the 17 species included in the study (Corrêa et al., 2005). In Eriocaulaceae, there are few studies on meiotic index and pollen viability, but *Syngonanthus mucugensis* Giul. and *Syngonanthus curralensis* Moldenke presented high pollen viability, 88.6% and 92.5%, respectively (Ramos et al., 2005).

Any abnormality in course of meiosis causes the formation of sterile gametes and low percentage of pollen viability (Jiang et al., 2011), leading to intraspecific reproductive barriers (Kaur & Singhal, 2019). However, the field observations reveal large populations. The population P2 was the most numerous (Table 3), despite presenting the lowest pollen viability value (Table 2). The results suggest that *S. caulescens* has the vegetative propagation as an important mechanism for the maintenance of its populations (Table 3). Five infraspecific taxons of *S. caulescens* are known (*S. caulescens* var. *caulescens* (Poir.) Ruhland,, *S. caulescens* var. *angustifolius* Moldenke,, *S. caulescens* var. *discretifolius* Moldenke, *S. caulescens* var. *douradensis* Moldenke, *S. caulescens* var. *gardenerianus* Moldenke), and, even within the varieties, a great morphological variability is recognized. This morphological and taxonomic variation could be attributed to the amplitude of vegetative propagation and the reproductive isolation of the populations. In accordance to our results, that suggests a significant clonal growth, Bento et al. (2010) also observed a wide clonal growth capacity in *Syngonanthus chrysanthus*, allowing its establishment and permanence in a previously colonized microenvironment. In addition, other genera of Eriocaulaceae, *Leiothrix* Ruhland and *Comanthera* L.B.Sm., also use this strategy (Coelho et al., 2007; Demetrio et al., 2014). According to Demetrio et al., (2014), *Comanthera nivea* (Bong.) L.R.Parra & Giul. presented a relation between clonality and performance of sexual reproduction because it increases the production of capitula. Even with low pollen grain production, a high number of capitula can be attractive to floral visitors.

In this study, despite the short observation time, we observed a considerable number of floral visitors belonging to Insecta and Arachnida, highlighting Diptera as the most representative in number of species and visits (Table 4; Figure 2). Studies in *Syngonanthus mucugensis*, *Syngonanthus curralensis* and *Syngonanthus elegans* Ruhland showed Coleoptera and Hymenoptera as the most representative floral visitors (Ramos et al., 2005). Although the flowers of *Syngonanthus* present floral traits associated with both anemophily (small flowers of separate sexes, placement of the anthers and stigmas above the perianth, small and numerous pollen grains, and a large number of staminate flowers in relation to pistillate flowers) and entomophily (the presence of odours, pigments on the flowers that absorb ultra-violet light, as well as pistillodes and nectariferous appendices), there was no evidence of wind pollination (Ramos et al., 2005). Entomophily is probably the only pollination system in *Syngonanthus* species (Ramos et al., 2005). Inflorescences of *Syngonanthus* are flat, floral rewards are easily accessible and there are no apparent morphological adaptations to specific visitors, being possible the visit of non-specialized insects with short mouthparts (Faegri & van der Pijl, 1979) such as Diptera, which had an expressive presence in populations P1B and P2.

Despite the occasions of ants acting as pollinators are rare and specific (Hickman, 1974; Dutton & Frederickson, 2012), we noted the representative presence of ants in all the populations. The ants are considered potential pollinators in several species, as in *Actinocephalus polyanthus* (Bong.) Sano, Eriocaulaceae, because they touch the anthers

by visiting the inflorescences, transferring small amounts of pollen to their bodies (Galitzki *et al.*, 2013). In *Paepalanthus lundii* Körn. was observed a relation between seed production and ant visits, proving that the ants were pollinators (Del-Claro *et al.*, 2019). Thus, when ants visit the staminate flowers in anthesis, they could move the pollen among inflorescences, acting as pollinators (Rosa & Scatena, 2007).

We also observed the presence of spiders, that construct their webs among the floral escapes of *S. caulescens*. Spiders, like ants, move through the floral capitula and can transfer pollen among the inflorescences, although the presence of pollen in the spiders bodies was not analyzed in this study. Spiders seem to use this strategy to obtain food. They wait for the visitors under the floral capitulum to trap them in their web and to perform the capture (Figure 2g-h). In other plant species, as the specialist *Arachis microspema* Krapov. W.C. Greg. & Valls (Fabaceae – Papilionoideae) and the generalist *Sphagnetocola trilobata* (L.) Pruski (Asteraceae), specimens of Thomisidae were observed performing the same hunting strategy, but there was no record of predation (Souza *et al.*, 2016). In Eriocaulaceae, spiders were also recorded in the umbels of *A. polyanthus*, but this behavior had been interpreted as harmful because they were predators of the other visitors (Galitzki *et al.*, 2013). Huey & Nieh (2017) demonstrated that the presence of spiders keep *Baccharis pilularis* DC. pollinators away.

The geographic and potential distributions of the suitable environments demonstrate that *S. caulescens* is widely distributed in Brazilian territory, but mainly found in the Cipó and Espinhaço Ranges, the diversification center of the family (Figure 3). Also, it is possible to visualize potential distribution of the species in Bolivia and Venezuela (Figure 3b), beyond Mexico and Central America (Giulietti & Hensold, 1990). The most species of Eriocaulaceae that had ecological niche evaluated, presents restricted distribution to a specific biome and environment (Giulietti *et al.*, 2016). These data can assist on finding new occurrences of *S. caulescens*, contributing to understand the ecological requirements of the species and its environmental risks. According to Giulietti *et al.* (2016), which investigated the distribution of the suitable environments of Eriocaulaceae in the Brazilian Amazon, modeling studies of species could be the key contribution towards the selection of priority areas for conservation or restoration in that region.

The savannahs grasslands in Brazil, called Pampa Biome, one of the occurrence areas of *S. caulescens*, is the Brazilian region with the smallest legally protected area, being influenced by agricultural expansions leading to loss of biodiversity and habitat fragmentation. The increasing fragmentation and degradation of the habitats, the pests, and diseases globalization, draining of wetlands for planting, and changing in climate conditions require coordinated action at local, regional, and national scales (Kramer & Havens, 2009) to promote the conservation of the species living in these environments. In other biomes where the species occurs, agricultural and livestock expansion is also a serious threat to the maintenance of natural environments.

Our study suggests that the studied populations of *S. caulescens* have the sexual reproduction compromised, since the meiotic index and pollen viability are very low during the full flowering phase. As a result, the species seems to depend on the vegetative propagation to maintain its populations. Our results also indicate an expressive number of floral visitors, demonstrating that important ecological interactions are happening. In addition, there are almost the entire Brazilian territory areas with the special environmental conditions for the occurrence of *S. caulescens*, agreeing with the collection records of the species.

## ACKNOWLEDGEMENTS

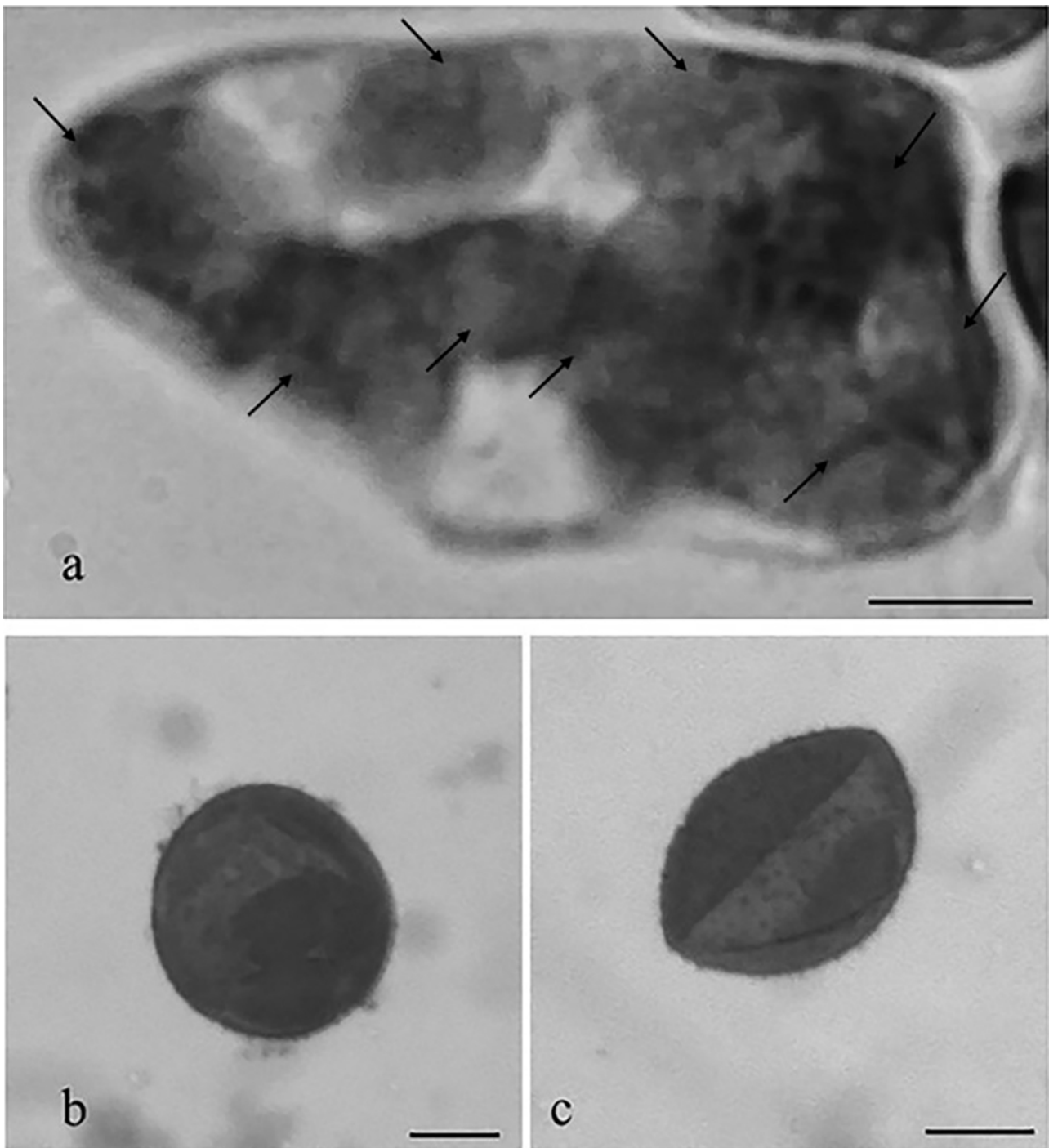
The authors thank to the *Programa de Educação Tutorial – PET Biologia UNIJUI (MEC/SESU)* for financial support, Vidica Bianchi for helping in the identification of the floral visitors and José Inácio Pereira, owner of the Taquari farm in São Francisco de Assis, Rio Grande do Sul State, Brazil.

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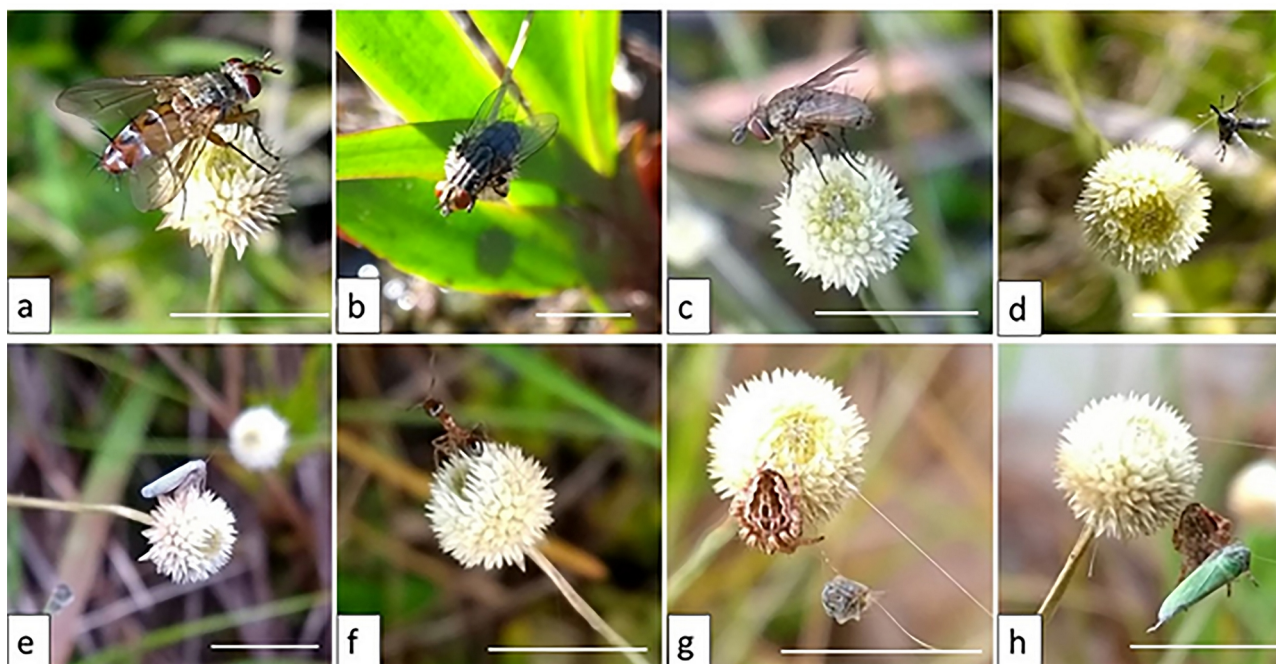
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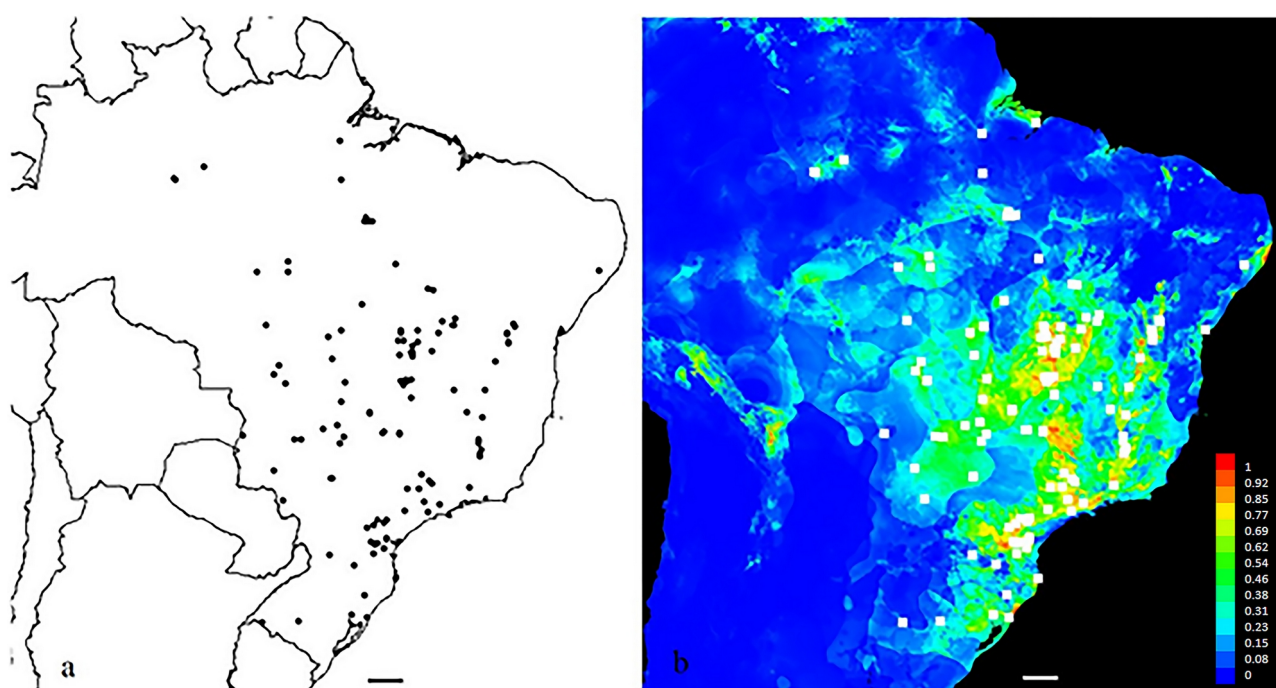
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**Figure 1:** Microspores of *Syngonanthus caulescens*. a. polyad with nine microspores P1A, 100x. Arrows indicate the microspores; b. viable pollen with content P1B, 40x; c. empty unviable pollen P1B, 40x. Scale = 10  $\mu$ m.



**Figure 2:** Floral visitors in *Syngonanthus caulescens*. a-b. Diptera, Muscidae; c. Diptera, Callyphoridae; d. Coleoptera, Curculionidae; e. Hemiptera, Sternorrhyncha; f. Hymenoptera, Myrmicidae; g-h. Araneae, Araneomorphae, capturing a Hemiptera, Sternorrhyncha. Scale = 0.5 cm.



**Figure 3:** Geographical distribution (a) and ecological niche modelling (b) of *Syngonanthus caulescens*. Scale = 200 km.

**Table 1:** Meiotic analysis in *Syngonanthus caulescens*.

Population	Total number of cells groups	Dyads/ Triads	Tetrads	Polyads (5-12 microspores)	Meiotic index (%)
P1A	85	6	16	63	18.8
P1B	66	19	15	32	22.7
P2	46	3	9	34	19.6
Total	197	28	40	129	20.3

**Table 2:** Pollen viability in *Syngonanthus caulescens*.

Population	Total pollen	Viable pollen	Unviable pollen	Pollen viability (%)
P1A	453	313	140	69.1 a
P1B	1,083	768	315	70.9 a
P2	544	20	524	3.6 b
Total	2,080	1,101	979	52.9

Average follow of the same letter not differ statically ( $\chi^2$ ,  $p < 0.05$ )

**Table 3:** Vegetative propagation capacity in *Syngonanthus caulescens*.

Population	Number of clumps	Number of individuals	Individuals average per clump	Number of vegetative individuals	Number of reproductive individuals
P1A	4a	21a	5.25±0.8a	5	16*
P1B	18b	88b	4.89±0.7a	44	44
P2	17b	102b	6±1.1a	72*	30
Total	39	211	5.41±0.46	121	90

Average followed of the same letter not differ statically ( $\chi^2$ ,  $p < 0.05$ ). \* Values differ statistically ( $\chi^2$ ,  $p < 0.05$ ).

**Table 4:** Floral visitors in *Syngonanthus caulescens*.

Population	Class/ Order	Suborder	Family	Subfamily	Species
P1A	Arachnida/ Araneae	Araneomorphae	-	-	sp.1
	Insecta/Diptera	Brachycera	Muscidae	-	sp. 2
	Insecta/Diptera	Brachycera	Syrphidae	-	sp. 3
	Insecta/Hymenoptera	-	Formicidae	Dolichoderinae	sp. 4
	Insecta/Hymenoptera	-	Formicidae	Dolichoderinae	sp. 5
	Insecta/Hymenoptera	-	Formicidae	Dolichoderinae	sp. 6
P1B	Arachnida/ Araneae	Araneomorphae	-	-	sp. 1
	Arachnida/ Araneae	Araneomorphae	-	-	sp. 7
	Insecta/Diptera	Brachycera	Callyphoridae	-	sp. 8
	Insecta/Diptera	Brachycera	Muscidae	-	sp. 9
	Insecta/Diptera	Brachycera	Muscidae	-	sp. 10
	Insecta/Diptera	Brachycera	Muscidae	-	sp. 11
	Insecta/Diptera	Brachycera	Syrphidae	-	sp. 12
	Insecta/Hymenoptera	-	Formicidae	Dolichoderinae	sp. 5



	Insecta/Hymenoptera	-	Formicidae	Formicinae	sp. 13
	Insecta/Hymenoptera	-	Formicidae	Ponerinae	sp. 14
	Insecta/Lepdoptera	-	-	-	sp. 15
	Arachnida/ Araneae	Araneomorphae	-	-	sp. 1
	Arachnida/ Araneae	Araneomorphae	-	-	sp. 16
	Insecta/Coleoptera	-	Crysmelidae	-	sp. 17
	Insecta/Coleoptera	-	Curculionidae	-	sp. 18
P2	Insecta/Diptera	Brachycera	Callyphoridae	-	sp. 8
	Insecta/Diptera	Brachycera	Muscidae	-	sp. 19
	Insecta/Diptera	Brachycera	Muscidae	-	sp. 20
	Insecta/Hemiptera	Sternorrhyncha	Psylloidae	-	sp. 21
	Insecta/Hymenoptera	-	Formicidae	Myrmicinae	sp. 22
	Insecta/Lepdoptera	-	-	-	sp. 23

**Table S1:** Collection data obtained on the Re flora Virtual Herbarium.

Voucher	Latitude	Longitude	Localization	Collection date
ALCB 16124	-19.28	-54.59	São Gabriel do Oeste/MS	Sep 16, 2002
ALCB 42843	-12.45	-41.46	Palmeiras/BA	Sep 26, 1994
ALCB 63645	-23.57	-48	Itapetinga/BA	Jan, 2013
ALCB 108640	-14.69	-42.55	Licínio de Almeida/BA	Feb 26, 2012
BOTU 21819	-12.85	-51.75	Barra do Garças/MT	Jan 01, 1972
BHCB 22188	-13.42	-48.04	Cavalcante/GO	-
BHCB 27604	-16.36	-45.06	São Romão/MG	Aug 23, 1990
BHCB 33387	-14.38	-47.53	Alto paraíso/GO	Mar 23, 1968
BHCB 56303	-21.62	-52.32	Santa Rita do Pardo/MS	Oct 14, 1998
BHCB 56304	-18.00	-43.39	São Gonçalo do Rio Preto/MG	Oct 01, 2000
BHCB 58082	-22.14	-44.09	Santa Rita de Jacutinga/MG	-
BHCB 158239	-23.53	0	Mogi das Cruzes/SP	Aug 11, 2007
BHCB 159090	-6.33	-50.16	Canaã dos Carajás/PA	Jun 19, 2012
CEN 17944	-13.46	-48.38	Minacú/GO	-
CEN 43604	-24.06	-49.06	Itapeva/SP	Aug 16, 2010
CEN 52815	-10.37	-46.58	Mateiros/TO	-
CEN 60588	-21.43	-47.07	Mococa/SP	Jan 23, 1997
CEN 60589	-24.56	-50.25	Tibagi/PR	Nov 28, 2013
CEN 61970	-17.69	-44.31	Joaquim Felício/MG	Jul 01, 1905
CEN 71783	-19.56	-51.88	Inocência/MS	Apr 21, 2004
CEN 83189	-15.92	48.00	Brasília/GO	-
CEN 83524	-14.15	-47.62	Alto Paraíso/GO	Apr 25, 1998
CESJ 11322	-15.78	-47.93	Brasília/GO	Jan 17, 1984
CGMS 6804	-21.62	-52.34	Santa Rita do Pardo/MS	Oct 14, 1998
CGMS 16637	-1.58	-48.2	Ilha de Itaparica/BA	Jan 01, 1953
CGMS 33029	-13.63	-41.87	Livramento do Brumado/BA	Dec 01, 1979
CPAP 22356	-16.05	-48.05	Gama/GO	Feb 01, 2001

Voucher	Latitude	Longitude	Localization	Collection date
EFC 10236	-25.55	-49.80	Balsa Nova/PR	Jan 01, 2007
ESA 33704	-17.68	-50.09	Edéia/GO	Oct 07, 2012
ESA 37979	-6.37	-50.38	Canaã dos Carajás/PA	May, 2012
ESA 73861	3.78	-61.72	Amajari/RR	Feb 18, 1967
ESA 81449	-13.08	-41.87	Piatã/BA	Sep 08, 1992
ESA 121234	-21.43	-47.01	Mococa/SP	Jan 23, 1997
FLOR 24906	-22.24	-47.14	Moji-Guaçu/SP	Mar 23, 1960
FLOR 57671	-29.88	-50.27	Osório/RS	Nov 29, 2014
FUEL 32717	-14.53	-52.31	Nova Xavantina/MT	Mar 28, 1997
FUEL 51548	-15.91	-51.58	Montes Claros de Goiás/GO	May 15, 2010
FUEL 54456	-25.17	-49.05	Campina Grande do Sul/PR	Feb 01, 2012
HAS 11279	-13.05	-38.73	Vera Cruz/BA	-
HCF 13200	-15.95	-55.03	Jaciara/MT	Sep 07, 2009
HCF 14316	-25.46	-49.77	Palmeira/PR	Dec 18, 2013
HCF 21023	-25.81	-49.21	Piraquara/PR	Mar 18, 2015
HCF 22387	-25.44	-50.04	Palmeira/PR	Jan 25, 1983
HJ 3989	-18.43	-51.99	Serranópolis/GO	Nov 20, 2004
HUCS 19984	-20.28	-43.50	Ouro Preto/MG	Jan 05, 1951
HRCB 27649	-19.28	-43.57	Santana do Riacho/MG	Jun 07, 1997
HRCB 44779	1.52	-51.85	Macapá/AP	Sep 30, 1949
HUEFS 27835	-17.11	-51.82	Caiapônia/GO	May 01, 1973
HUEFS 72948	-13.6	-41.8	BA	Sep 06, 2003
HUEFS 73865	-6.35	-49.84	Canaã dos Carajás/PA	Feb 05, 2013
HUEFS 75386	-15.87	-48	Distrito Federal/GO	Mar 28, 2000
HUEFS 82887	-12.81	-51.77	Base Campo/MT	May 12, 1969
HUEFS 107148	-16.06	-55.07	Jaciara/MT	Mar 06, 2009
HUEFS 128981	-25.42	-50.00	Palmeira/PR	Jan 25, 1983
HUEFS 129828	-19.03	-48.26	Uberlândia/MG	Apr 27, 2014
HUEFS 130373	-13.00	-38.78	Vera Cruz/BA	-
HUEFS 169167	-18.61	-52.85	Costa Rica/MS	Oct 26, 2004
HUEFS 188322	-21.71	-46.54	Poço de Caldas/MG	Jul 01, 1942
HUEFS 195929	-15.92	-48.00	Brasília/GO	May 02, 2007
HUFU 21298	-18.91	-48.27	Uberlândia/MG	Sep 24, 1999
HUFU 59029	-28.59	-50.38	Bom Jesus/RS	Jan 13, 1947
HUFU 64109	-14.12	-47.52	Alto Paraíso/GO	May 24, 1993
HUFU 67574	-24.38	-49.85	Jaguariaíva/PR	-
IAC 32403	-24.27	-49.28	Itararé/SP	May 04, 2006
IAC 32423	-24.13	-49.69	Sengés/PR	Nov 22, 2016
IAC 49842	-3.067	-59.99	Manaus/AM	Dec, 1850
ICN 124396	-12.38	-45.73	Luis Eduardo Magalhães/BA	Mar 12, 2010
ICN 160870	-12.51	-45.09	São Desidério/BA	Jan 01, 2013
ICN 174787	-0.88	-48.68	Ilha do Marajó/PA	-

Voucher	Latitude	Longitude	Localization	Collection date
INPA 2367	-12.81	-51.77	MT	-
INPA 96651	-14.38	-47.53	Alto Paraíso/GO	Mar 23, 1968
INPA 113187	-9.38	-54.91	Itaituba/PA	Apr 25, 1983
INPA 250302	-29.88	-50.27	Osório/RS	Dec 15, 2014
JPB 2345-B	-22.95	-55.22	Amambai/MS	Mar 20, 2005
MBM	-8.77	-54.94	PA	Nov 04, 1977
MBM	-25.46	-49.76	Palmeira/PR	Dec 18, 2013
MBM 54876	-22.28	-47.88	Itirapina/SP	Jul, 2015
MBM 129101	-25.48	-49.76	Palmeira/PR	May 12, 2014
MBM 194946	-18.66	-52.86	Costa Rica/MS	Oct 26, 2004
MBM 198061	-21.15	-55.83	Nioaque/MS	Sep, 2003
MBM 258258	-12.89	-48.22	São Salvador do Tocantins/TO	Jul 02, 1905
MBM 285750	-12.52	-45.10	São Desidério/BA	Apr, 2008
MO 3050514	-13.00	-46.00	Correntina/BA	1992
NY 2224	-14.15	-47.6	Alto Paraíso/GO	Apr 25, 1998
NY 2228	-23.12	-45.88	São José dos Campos/SP	Feb 10, 1962
NY 2238	-22.24	-47.14	Moji- Guaçu/SP	Mar 23, 1960
NY 2241	-14.97	-55.45	Jauru/MT	Mar 07, 2002
NY 2247	-23.65	-46.62	São Paulo/SP	Jun 26, 1944
NY 538205	-18.94	-49.25	Ituiutaba/MG	Apr 10, 2013
NY 538206	-26.17	-49.77	Mafra/SC	Jan 26, 1953
NY 538250	-12.86	-47.22	Paraná/TO	-
NY 572013	-25.30	-49.89	Campina Grande do Sul/PR	Feb 01, 2012
NY 822851	-28.58	-50.38	Bom Jesus/RS	Jan 05, 1947
NY 898227	-18.20	0	Diamantina/MG	Jun 09, 2012
NY 898230	-19.95	-43.41	Santa Barbara/MG	Apr 14, 1933
NY 898234	3.83	-61.67	Alto Alegre/RR	Dec 23, 1987
NY 898235	-8.85	-48.52	Guaraí/TO	Mar 19, 1968
NY 898236	-16.80	-47.6	Cristalina/GO	Nov 02, 1965
NY 898237	-16.40	-43.21	Salinas/MG	Feb 14, 1969
NY 898238	-20.13	-43.49	Barão de Cocais/MG	Jan 22, 1971
NY 898246	-15.43	-55.77	Chapada dos Guimarães/MT	Oct 21, 1973
NY 898250	-25.47	-49.65	Balsa Nova/PR	Jan 16, 2012
NY 898255	-25.45	-49.65	Balsa Nova/PR	Feb, 2014
NY 898266	-12.15	-44.93	Barreiras/BA	Jul 14, 1984
NY 898273	-13.2	-52.57	Garapú/MT	Sep 30, 1964
NY 2086674	-9.25	-36.43	Quebrangulo/AL	Feb 02, 2014
NY 2086676	-13.55	-47.25	Alto Paraíso/GO	May, 2002
NY 2086677	-12.94	-48.20	Palmeirópolis/TO	-
NY 2086678	-12.49	-56.26	Tapurah/MT	Jan 01, 1998
NY 2086686	3.85	51.80	Macapá/AP	Sep 30, 1949
NY 2086687	-12.58	-41.46	Lençóis/BA	Aug, 2011

Voucher	Latitude	Longitude	Localization	Collection date
NY 2086690	-12.86	-47.22	Paraná/TO	-
PACA-AGP 7965	-18.89	-49.21	Ituiutaba/MG	Apr 10, 2013
PACA-AGP 9592	-15.76	-47.64	Brasília/GO	-
PACA-AGP 9717	-15.87	-47.85	Brasília/GO	Feb, 2007
PACA-AGP 31472	-25.46	-49.76	Palmeira/PR	May 12, 2014
R 181756d	-29.75	-51.18	São Leopoldo/RS	Dec, 1941
RON 10901	-25.46	-49.76	Palmeira/PR	May 12, 2014
SP 10190	3.83	-61.67	Alto Alegre/RR	Dec 23, 1987
SPF 215475	-22.26	-47.82	Itirapina/SP	Jul, 2015
SPSF 21376	-9.27	-62.91	Itapua do Oeste/RO	Apr 19, 2015
UB	-15.86	-48	Distrito Federal/GO	Oct 20, 1975
UB 3586	-13.76	-47.5	Alto Paraíso de Goiás/GO	Jul 31, 1994
UB 10915	-9.34	-56.78	Paranaíta/MT	Feb 08, 2012
UB 78864	-14.09	-46.3694	Posse/GO	Jan 27, 2005
UB 78903	-17.77	-50.10	Edéia/GO	Oct 04, 2012
UB 78905	-30.11	-54.32	São Gabriel/RS	Nov 16, 2015
UB 78999	-14.29	-48.33	Niquelândia/GO	May 28, 1996
UB 163898	-13.61	-41.89	Rio de Contas/BA	Oct 05, 2003
UB 191402	-13.57	-41.83	Rio de Contas/BA	Aug 20, 2007
UB 210974	-17.72	-50.07	Edéia/GO	Oct 10, 2012
UEC 31530	3.83	-61.67	Alto Alegre/RR	Dec 23, 1987
UEC 38684	-13.01	-38.78	Vera Cruz/BA	-
UEC 48414	-26.22	-52.43	Clevelândia/PR	May 01, 1966
UEC 103227	-15.79	-47.94	Brasília/GO	Sep 20, 1977
UEC 173955	-27.59	-48.54	Florianópolis/SC	Dec 28, 1994
UEC 173956	-24.26	-49.29	Itararé/SP	May 04, 2006
UEC 196891	-23	-46 83	Itatibá/SP	Jul 22, 1996
UFMT 9447	-13.54	-41.83	Rio de contas/BA	Feb, 2007
UFMT 33190	-11.30	-50.56	São Félix do Araguaia/MT	Jan 01, 1998
UFMT 33192	-21.93	-46.38	Caldas/MG	1952
UFMT 39964	-10.39	-46.29	Caxambu/MG	Aug 01, 2010
UFMT 40647	-25.17	-49.09	Campina Grande/PR	Feb 01, 2012
UNOP 3361	-29.88	-50.27	Osório/RS	Dec 15, 2014
UPCB	-19.53	-43.52	Nova União/MT	Aug 11, 2013